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JC682 U.S. PTO 08/25/99

UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	9341-018-999	Total Pages	49
First Named Inventor or Application Identifier			
Michael Meyrick Burrell et al.			
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APPLICATION ELEMENTS
See MPEP chapter 600 concerning utility patent application contents.

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   - -Descriptive title of the Invention
   - -Cross Reference to Related Applications
   - -Statement Regarding Fed sponsored R&D
   - -Reference to Microfiche Appendix
   - -Background of the Invention
   - -Brief Summary of the Invention
   - -Brief Description of the Drawings *(if filed)*
   - -Detailed Description of the Invention (including drawings, *if filed*)
   - -Claim(s)
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   Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33 (b).
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Modification of Plant Fibres

This invention relates to the modification of the morphology of plant fibre cells. The invention is exemplified by methods of using genetic constructs for the modification of, in particular, but not exclusively, *Eucalyptus* fibres, for example.

The primary product of the forestry industry is considered to be wood, although more fundamentally it could be defined as fibre. The industry supplies a wide range of feedstocks to the solid wood and pulp/paper industries who produce a multiplicity of products. The forester must therefore seek to cater for the competing needs of these industries, and even within the individual industries, there is a range of different requirements. For example, different paper grades require different qualities in the starting material.

Forestry-based operations depend upon a balance between the capability of the forester to supply the processor with fibre having specific properties, and the ability of the processor to modify his process and so accommodate the available feedstock. The design and operation of processing plants are influenced by the wood (fibre) properties of the feedstock.

Notwithstanding these specific demands, fibre uniformity and strength are common requirements for most industrial uses, and hence the fibre supplied by the forester must be capable of delivering these properties to the processor.

In pulp manufacture, for example, strength characteristics are determined in part by fibre length. Increased fibre length leads to the production of paper with increased strength. Bond strength is attributed to contact between the fibres and the adhesion capabilities of the surfaces, which are dependent upon fibre length, perimeter and coarseness. Also, during the manufacturing process, increased fibre length increases the strength of wet webs enabling easier handling (Seth, 1995).

However, long fibres are not desirable for all applications. In some cases, shorter fibres are preferable, such as in the production of smooth-surfaced papers.

Fibre properties differ between species, and consequently particular species have been limited historically to particular applications. Fibres from hardwood species are generally much shorter than those from softwoods. This results in the production of pulp and paper with desirable surface characteristics such as smoothness and brightness, but with low strength characteristics. In practice, where a single species providing fibre with an appropriate combination of characteristics has not been available, the mixing of long

and short fibres from different species is used. If a single source were available, possessing the desirable characteristics plus optimal fibre length, this would be of great benefit to the processor. Some common species and their fibre lengths are exemplified in Table 1 below.

Table 1

Fibre Lengths of Various Tree Species

Species	**Fibre Length (mm)**
Loblolly Pine	3.5 - 4.5
Western Hemlock, Western Spruce	2.5 - 4.2
Southern Hardwood	1.2 - 1.4
Northern Hardwood	1.0 - 1.2
Eucalyptus	0.8 - 1.0
White Oak	0.59
Sweetgum	0.48
Aspen	0.35

Eucalyptus trees represent the largest sources of fibres used globally in the paper industry (Bamber 1985; Ranatunga, 1964), and world-wide, there are an estimated ten to fifteen million hectares of land planted with *Eucalyptus* (Verhaegen and Plomion 1996). The major advantage of Eucalypts is their very high growth rates and ability to grow in a wide range of conditions, both tropical and temperate.

However, *Eucalyptus* fibres are significantly shorter than those from other, once more popular, sources of fibre such as pine. Thus papers that are made from *Eucalyptus* pulp are often weak and usually require reinforcement with longer fibres from other sources increasing the production costs. If trees could be produced with longer fibres, this would be a considerable advantage to the paper industry, increasing the quality of the raw materials for pulp and paper synthesis.

Through tree breeding it is possible to achieve some modification of fibre characteristics. For example, interspecific triploid hybrids of poplar have been developed which have longer fibres than the parental species.

Genetic variation in fibre properties is also evident within species. Fibre characteristics are controlled by a complex set of genetic factors and are not easily amenable to classical breeding methods. Therefore, existing genetic variation has not been exploited significantly in tree breeding programmes. Whilst knowledge is now being accumulated on the heritability of wood properties, previously these were not often considered as important as growth characteristics and were sometimes sacrificed in pursuit of the latter. In some instances, growth rate is negatively correlated with fibre characteristics, though this does not always hold true (e.g. in Eucalypts), and breeding efforts are now being made to capture the benefits of both.

In many cases fibre properties are sufficient for the end product, and improvement is considered unnecessary. For example, increasing fibre length beyond 2mm causes little increase in tear strength or tensile strength, and many softwood fibres are commonly around 3mm long, i.e. greater than the minimum for desired strength. However, fibres in juvenile wood tend to be shorter and there is an increased usage of juvenile material through a reduction in rotation times. Hence, there is scope for improvement even in those species which commonly yield long fibres.

From the perspective of the pulp and paper industry, fibres are specific types of plant cell walls that have been subjected to a range of treatments to remove all contents and most non-cellulosic wall components (Stewart *et al*, 1994). In woody plants the fibres are made up of dead cell wall material. In order to produce longer fibres it is necessary to have longer living cells during growth, before fibre formation.

The cell wall can be envisaged as a complex network of cellulose microfibrils linked together by noncovalent interactions with matrix polymers (Carpita and Gibeaut, 1993). The microfibrils are coated by a mixture of hemicelluloses which form extensive hydrogen-bonded interactions with the surface of the microfibrils. Coextensive with this is another

network formed from various pectins which are held together largely by ionic linkages (McQueen-Mason, 1995).

To allow cells to grow and enlarge the wall components must loosen to enable slippage of the polysaccharides and proteins within the matrix (Cosgrove, 1993). Extension of the cell is then driven by the internal turgour pressure of the cell, which is considerable. The degree of extension during cell growth is controlled by the mechanical properties of the cell wall, which result from their composition and from the orientation of wall fibrils and structural polymers.

The control of cell wall extension is closely regulated by the plant to facilitate growth control and morphogenesis. The ultimate agents of control are enzymes located in the wall itself. If plants express cell wall "loosening" enzymes in their walls, then it seems likely that these enzymes can regulate cell growth. Altered levels of expression can thereby cause increased or reduced cell growth and fibre length. Changes in cell wall texture may also be produced.

One class of cell wall proteins are the Expansins. Expansins induce the extension of plant walls, and at present are the only proteins reported with demonstrated wall-loosening activity. Expansins were first isolated from cucumber hypocotyl cell walls by McQueen-Mason *et al* (1992) and characterised by their ability to catalyse wall loosening in an *in vitro* rheological assay.

The mode of action of expansins is believed to be by weakening the noncovalent bonding between the cellulose and hemi-cellulose, with the result that the polymers slide relative to one another in the cell wall (Cosgrove 1996). The precise biochemical action of expansins is unclear, although it is known that their effects are not due to exoglycanase or xylogucan endotransglycosylase activity (McQueen-Mason *et al*, 1992, McQueen-Mason & Cosgrove, 1993). Expansins appear to disrupt hydrogen bonding between cellulose microfibrils and hemicelluloses. The process enables wall loosening without any degradation of the polymers or an overall weakening of wall structure during expansion. Consistent with this mechanism, expansins have been shown to weaken cellulosic paper, which derives its mechanical strength from hydrogen bonding between cellulose fibres (McQueen-Mason and Cosgrove, 1994).

Expansins are able to restore the ability of isolated cell walls to extend in a pH dependent manner (McQueen-Mason and Cosgrove, 1995) and may be responsible for the phenomenon of "acid growth" in plants (Shcherban *et al*, 1995). Expansin proteins have been characterised in cucumber hypocotyls (McQueen-Mason *et al*, 1992), oat coleoptiles (Li *et al*, 1993), expanding tomato leaves (Keller and Cosgrove, 1995) and rice internodes (Cho and Kende, 1997).

Expansin cDNAs have been isolated and characterised from a number of plants and it is now evident that expansins exist as a multi-gene family showing a high level of conservation between species. cDNAs with high degrees of homology have been identified from collections of anonymous Expression Sequence Tag (EST) cDNAs from *Arabidopsis* and rice. These EST cDNAs exhibit a high degree of homology at the level of protein sequence (60-87%) indicating that expansin structure is highly conserved (Shcherban *et al* 1995). Expansins show no sequence similarity to other known enzymes, although they do have sequence similarities to some pollen allergens (Shcherban *et al*, 1995). Recently Cosgrove *et al* (1997) have shown that pollen allergens from maize also possess considerable expansin activity.

If plants can be modified to over-express expansins in their walls, then it would be expected that these plants will exhibit a marked increase in cell extension or growth. Conversely, a reduction in the expression of expansins should lead to a reduction in cell growth. It is therefore surprising that constitutive expression of expansin in eucalypts results in a reduction in height and internode length.

One approach to modifying the expression of expansins is via the introduction of recombinant DNA sequences into the plant genome. Several methods can be used to introduce

foreign DNA into plant cells (see review by Weising *et al*, 1988; Miki and Iyer, 1990 and Walden 1994). *Agrobacterium tumefaciens*-mediated gene transfer is probably the most widely used and versatile of these methods (Walden, 1994).

Genetic modification experiments directed towards changing the wood and paper quality of trees has been investigated by other workers, particularly focusing on the lignin pathway in cells and lignin content in the final paper product (Hawkins and Boudet, 1994; Grima-Pettenati, *et al*, 1993; Poeydomenge *et al*, 1993; Boudet *et al*, 1995 and Hibino *et al*, 1994). The aim of the present invention differs in that it seeks to provide a means of controlling fibre growth and cell wall morphology.

An object of the present invention is to provide a method whereby trees can be modified to produce fibres of a desired length for specific applications. This will enable the forester to control the quality of his product. In addition it will enable the forester to produce a wide range of fibre types from a single or small number of species which can be selected as being ideally suited for cultivation in that particular site. This will result in both the economy of employing a single uniform silvicultural regime, and the flexibility of producing which ever type of fibre is required at a particular time.

The invention also provides a means of producing fibre of specific type from trees at particular periods in their growth cycle. For example, the production of long fibres from juvenile trees can be achieved, thereby accelerating the time to harvest of the crop.

This is achieved by firstly isolating and characterising expansin gene sequences from heterologous and homologous species and then reintroducing these genes into trees so as to alter expansin levels in the transgenic trees using the well known over-expression, co-suppression (described by DNAP in their European Patents Nos. 0465572 and 0647715) and anti-sense knockout strategies. This will lead to the cultivation of trees more suitable for paper production.

The present invention provides a nucleic acid coding sequence encoding a gene capable of modifying the extension of fibre cell walls, the nucleic acid coding sequence being one or more of SEQ.ID. Nos. 1-6 hereof.

The present invention also provides a method of transforming trees to modify the fibre characteristics in trees, the method comprising stably incorporating into the plant genome a chimaeric gene comprising a promoter and a nucleic acid coding sequence encoding a gene capable of modifying the extension of fibre cell walls, and regenerating a plant having an altered genome.

The present invention also provides trees having therein a chimaeric gene comprising a promoter and a nucleic acid coding sequence capable of modifying the extension of fibre cell walls.

The present invention also provides a chimaeric gene capable of modifying the extension of cell walls, said chimaeric gene comprising a promoter and a nucleic acid coding sequence encoding a gene capable of modifying the extension of fibre cell walls, said nucleic acid coding sequence being one or more of SEQ. ID. Nos. 1-6 or the cucumber Ex 29 coding sequence, or a sequence which has sufficient homology to hybridise to any one of SEQ. ID. Nos. 1-6 or cucumber Ex 29 under medium stringency conditions.

Preferably the chimaeric gene further comprises a terminator.

Constructs having the DNA structural features described above and trees incorporating such constructs and/or chimaeric genes according to the invention are also aspects of the invention.

Plant cells containing chimaeric genes comprising a nucleic acid coding sequence capable of modifying the extension of fibre cell walls are also an aspect of this invention, as is the seed of the transformed plant containing chimaeric genes according to the invention.

The chimaeric gene may comprise the nucleic *acid coding* sequence as it exists in the genome, complete with endogenous promoter, terminator, introns and other regulatory sequences, or the nucleic acid coding sequence, with or without introns, may be combined with a heterologous promoter, terminator and/or other regulatory sequences.

The promoter may be a constitutive promoter, such as the cauliflower mosaic virus 35S promoter (CaMV35S), the cauliflower mosaic virus 19S promoter (CaMV19S) or the nopaline synthase promoter, a tissue specific promoter, such as the *rol*C, patatin or *petE* promoters, or an inducible promoter, such as AlcR/AlcS. Other suitable promoters will be known to those skilled in the art.

The nucleic acid sequence, or parts thereof, may be arranged in the normal reading frame direction, i.e. sense, or in the reverse reading frame direction, i.e. antisense. Up or down regulation of the activity of the expansin protein or gene encoding therefor using sense, antisense or co-suppression technology may be used to achieve alteration in the length of fibre cell walls.

Preferably the nucleic acid sequence encodes one or more of the class of proteins known as expansins. More preferably the nucleic acid sequence is derived from *Eucalyptus* or cucumber.

The nucleic acid sequence may advantageously be one or more of SEQ. ID. Nos. 1-6 hereof. Alternatively, the nucleic acid sequence may be the cucumber expansin sequence cucumber Ex29 (GenBank Accession No. U30382 - known as Cs-EXP1). The sequence is also described in Shcheraban *et al* (1995).

Alternatively, the nucleic acid sequence may be a sequence which has sufficient homology to hybridise to any one of SEQ. ID. Nos. 1-6 or cucumber Ex29 under medium stringency conditions (washing at 2x SSC at 65°C).

Preferably the nucleic acid sequence is an mRNA or cDNA sequence, although it may be genomic DNA.

Trees which may suitably be transformed using the inventive method include Eucalypts, Aspen, pine, larch.

The nucleic acid sequence may be introduced by any of the known genetic transformation techniques such as *Agrobacterium tumefaciens* mediated transformation, *Agrobacterium* rhizogenes mediated transformation, biolistics, electroporation, chemical poration, microinjection or silicon-fibre transformation, for example.

In order that the invention may be easily understood and readily carried into effect, reference will now be made, by way of example, to the following Figures, in which:-

Figure 1a is a diagrammatic representation of the coding sequence for cucumber Ex29 cloned between the cauliflower

mosaic virus 35S promoter and nos terminator in the vector pDE326;

Figure 1b is a diagrammatic representation showing the insert from Figure 1a between the EcoR I and Hind *III* restriction sites introduced into a modified Ti plasmid pDE 1001 to produce pDE/EXP29, and

Figure 1c is a diagrammatic representation showing the insert from Figure 1a between the EcoR I and Hind *III* restriction sites introduced into a modified Ti plasmid p35GUSINT to produce pATC/EXP29; and

Figure 2 is a diagrammatic representation showing an insert containing SEQ. ID. No. 1 between the EcoR I and Hind *III* restriction sites introduced into a modified Ti plasmid p35GUSINT to produce pATC/SEQ. ID. No.1.

EXAMPLE 1

Isolation of novel expansin sequences from *E. grandis* stem tissue

RNA extraction from cucumber hypocotyls. Seeds of cucumber (*Cucumis sativus* L., cv Burpee pickler, from A.W. Burpee, Westminster, Penn, USA) were sown on water-soaked capillary matting (Fordingbridge Growers Supplies, Arundel, W. Sussex, UK) in plastic trays (35cm x 25cm x 6cm) and germinated in the dark at 27°C. After 4 days the etiolated seedlings were harvested under green light by excising the upper 20mm of the

hypocotyl into liquid nitrogen and grinding to a fine powder in a pestle and mortar that had previously been chilled at -80°C. Total RNA was extracted in a hot phenol/lithium chloride buffer according to the procedure of Verwoerd *et al* (1989).

RNA extraction from *Eucalyptus grandis*. *E.grandis* seeds were sown on trays (35cm x 25cm x 6cm) of Levington's F2 compost (Levington Horticulture Ltd., Ipswich, Suffolk, UK) and germinated in a greenhouse (18-24°C, at a light intensity of approximately 10,000 lux, and 16 hours of daylight). After 8 weeks the seedlings were transferred to individual pots, and then repotted as necessary (approximately every 6-7 weeks). Growing stem tissue was harvested from the last 40-50mm of branch tips into liquid nitrogen. Immature leaves, usually the youngest two from growing branch tips, were also harvested directly into liquid nitrogen; roots were washed in several bowls of tap water, rinsed with distilled water and then growing tips were excised into liquid nitrogen. RNA was extracted as described by Pawlowski *et al* (1994) using a protocol especially modified for the extraction of RNA from plants containing high levels of phenolic compounds.

Poly(A^+) mRNA isolation from total RNA extracted from *E.grandis* stem tissue. Poly(A^+) mRNA was isolated from total RNA using either push (Stratagene, Cambridge, UK) or spin

oligo(dt) columns (Clontech Laboratories, Inc. CA., USA) and following the supplier's instructions and recommendations.

RT-PCR and Sequencing. The nucleic acid sequence of expansins show a considerable extent of divergence. However two regions with a reasonable degree of consensus were identified and used to synthesise two oligonucleotide primers of low complexity (see Table 2).

Total RNA was extracted from young stem tissue and Poly(A^+) mRNA isolated using oligo(dt) columns as described above. 1μg of mRNA was used in a PCR experiment (50°C annealing temperature, 30 cycles, hot start) with the two expansin consensus primers and Taq DNA polymerase (Promega UK Ltd.).

Table 2

Sequence of Consensus Expansin Primers

	Sequence (5' -3')
P.1 (SEQ. ID. No. 7)	ATGGIGGIGCNTGYGGNTA
P.2 (SEQ. ID. No. 8)	TGCCARTTYTGNCCCCARTT

Key: Y=C or T, N=A or G or C or T, R=A or G, I=Inosine

cDNA Library Construction. For first strand cDNA synthesis 1μg of mRNA was used in a reaction with 0.15μg OG1 oligo dt primers and AMV Reverse Transcriptase (9 units/μl, Promega UK Ltd., Southampton, UK).

The library was constructed in the Lambda ZAP II vector (Stratagene, Cambridge, UK), following the supplier's instructions.

Using the methods described, transformed clones were isolated by blue-white colony selection on agar plates following the methods described by the supplier (R&D Systems). Twenty white ("positive") colonies were selected and sequenced. Of these, six were identified as containing sequences that had similarities with other known expansin sequences using a basic BLAST search provided by NCBI. The putative transcripts were all around 450 bps in size (determined by PCR and gel electrophoresis). PCR products were sequenced using a forward primer and the sequences identified as SEQ.ID. Nos. 1-6 were obtained.

EXAMPLE 2

Northern Analysis

Total RNA was isolated from the stem, leaves and roots of *E. grandis* as described above. 6μg of RNA in 20μl DEPC H_2O was denatured in a equal volume of denaturing solution (50% formamide, 2x TBE) and run on a standard 1.5% agarose gel at 75 volts for 200 min. RNA from the gel was transferred onto "Zeta-Probe" GT Genomic Tested Blotting Membranes (Biorad Laboratories, California, USA) by capillary transfer. Partial *E.grandis* expansin sequences generated by RT-PCR from stem

mRNA (as described above) were used for 32P-random prime labelling and hybridised to the transferred RNA following the membrane supplier's recommended methods (Biorad Laboratories).

Example 3

Preparation of Exp29 transformation vector.

RNA extraction from cucumber hypocotyls. Seeds of cucumber (*Cucumis sativus* L., cv Burpee pickler, from A.W. Burpee, Westminster, Penn, USA) were sown on water-soaked capillary matting (Fordingbridge Growers Supplies, Arundel, W. Sussex, UK) in plastic trays (35cm x 25cm x 6cm) and germinated in the dark at 27°C. After 4 days the etiolated seedlings were harvested under green light by excising the upper 20mm of the hypocotyl into liquid nitrogen and grinding to a fine powder in a pestle and mortar that had previously been chilled at -80°C. Total RNA was extracted in a hot phenol/lithium chloride buffer according to the procedure of Verwoerd *et al* (1989).

Vector construction. The coding sequence for cucumber Ex29 (GenBank Accession No. U30382; known as Cs-EXP1, and Shcherban *et al* 1995) was generated by RT-PCR and cloned between the Cauliflower Mosaic Virus 35S promoter and *nos* terminator (see Figure 1a) into pDE326, a vector kindly donated by Dr. Jürgen Denecke of York University. After insertion of the Ex29 expansin sequence the inserts were

sequenced to check for correct in frame insertion by sequencing using a primer located within the 35S promoter region.

Inserts containing the 35S promoter, Ex29 sequence and *nos* terminator were cut between the EcoRI and Hind*III* restriction sites and inserted into modified Ti plasmids to produce transformation constructs. Two modified Ti plasmids were used: pDE1001 (Denecke *et al*, 1992 or Shcherban *et al* 1995) provided by Dr. Jürgen Denecke and p35GUSINT (Vancanneyt *et al*, 1990). The plasmids produced containing the insert were referred to as pDE/EXP29 (pDE1001 + Ex29) (see Figure 1b) and pATC/EXP29 (p35GUSINT + Ex29) (See Figure 1c), acknowledging the source of the plasmids.

Plasmids were transferred into *E.coli* by standard procedures; *E.coli* strains were grown on LB plates (incubated at 37°C and stored at 4°C) or in LB medium with the appropriate antibiotic for positive selection.

The constructs were introduced into *Agrobacterium* via direct DNA transformation or by tri-parental mating using the *E.coli* mobilisation function strain HB101 (pRK2013) (Figurski and Helinski 1979).

Two strains of *Agrobacterium tumefaciens* were used. A C58 strain (C58C1(pGV2260) Deblaere, R. *et al* 1985) kindly donated by Dr. Jürgen Denecke, and EHA105 (Hood *et al* 1993). *Agrobacterium* were grown on LB plates (incubated at 27°C and

stored at 4°C) or in LB medium with the appropriate antibiotic for positive selection.

Agrobacterium tumefaciens EHA105 pATC/EXP29 was deposited by Advanced Technologies (Cambridge) Limited of 210 Cambridge Science Park, Cambridge CB4 4WA, under the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for the purposes of Patent Procedure at the National Collection of Industrial and Marine Bacteria (NCIMB), 23 St. Machar Street, Aberdeen, Scotland on 25 August 1998 under Accession No. NCIMB 40968. The micro-organism is *Agrobacterium tumefaciens* : strain EHB105, containing pATC/EXP29. The cDNA for cucumber EX29 was inserted into disabled/disarmed pBIN19 (Bevan, 1984) with the 35S cauliflower mosaic virus promoter and nos terminator. The plasmid was then transferred into the *Agrobacterium* strain EHA105. The construct is useful for altering the extension of fibre cell walls.

EXAMPLE 4

Plant Transformation

Young leaves were dissected under sterile conditions, from approximately 4 week old *E.grandis* cultures grown in Magenta boxes (7cm x 7cm x 13cm) on LS media at 25°C, in a growth room in our tissue culture laboratory and used for *Agrobacterium*-mediated infection (Horsch, Fry, Hoffman,

Eichholtz, Rogers, and Fraley 1985). Inoculated tissue was left to co-cultivate for 4d on LS media (plus 20g/l glucose, 0.7% agarose, 0.01mM Zeatin a 1μM NAA) in diffuse light in a growth room, conditions as before. Transformants were selected on 50mg/l kanamycin and 250mg/l claforan.

Two constructs for plant transformation were prepared and introduced into two strains of *Agrobacterium*, C58 and EHA105 to produce C58 containing pDE + Ex29, C58 containing pATC + Ex29 and EHA105 containing pATC + Ex29. Each construct-containing strain was used to inoculate 400 leaves dissected from *E.grandis* tissue (on two separate occasions, each time inoculating 200 leaves).

The transformation experiments were repeated with a further 240 leaves, inoculated with EHA105 containing pATC + Ex29 to increase the amount of possible transformants obtainable.

From the original batch of inoculated tissue with EHA105, 25 plants were grown in the greenhouse and the properties of the shoots determined.

The introduction of the expansin coding sequence attached to the 35SCAmV promoter seems to have caused a reduction in the overall height of the plants from a mean control value of 603mm in the control plants to 546mm in the transformed plants. Of the survivors of the 25 plants, 4 control and 13 transgenic plants were included in this analysis. This

reduction in height is associated with a change in internode length as analysed in the table below. A Chi square analysis of the data in Table 3 indicates that the two populations of plants are significantly different at a value of P<0.01.

TABLE 3

Class of Internode Length (mm)	**Number of Internodes in class**		**% of internodes in class**	
	Control	**Expansin**	**Control**	**Expansin**
10	1	7	3	6
20	10	28	28	24
30	9	28	28	24
40	4	21	11	18
50	1	9	3	8
60	6	12	17	10
70	2	7	6	6
80	3	4	8	3
>80	01			

From the data it is clear that a modification in the level of expansin activity in the tree can be used to produce a required effect. In order to increase the growth it may be necessary to use down regulation technology, e.g. expression of the reverse or complementary strand of the expansin sequence, or a partial sense expansin sequence, in order to increase the fibre length.

EXAMPLE 5

Sequences SEQ. ID. Nos. 1-6 were each introduced into pATC in both orientations, i.e. antisense and sense orientation, and were used to transform Eucalypts and tobacco using the same methodology as described in Examples 3 and 4. Figure 2 shows the plasmid pATC/SEQ. ID. No. 1 in sense orientation, as a representative of the plasmids used in the transformation. Any suitable transformation vector can be used.

It was found that the introduction of the novel expansin sequences produced transformed plants different from the control plants.

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BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

NAME AND ADDRESS OF DEPOSITOR	
Advanced Technologies (Cambridge) Ltd Unit 210 Cambridge Science Park Cambridge CB4 4WA	**INTERNATIONAL FORM** **RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT** **issued pursuant to Rule 7.1 by the** **INTERNATIONAL DEPOSITARY AUTHORITY** **identified at the bottom of this page**

I IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR.	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
Agrobacterium tumefaciens (EHA105 pATC/EXP29)	NCIMB 40968

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by.

☐ a scientific description

☒ a proposed taxonomic designation

(Mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I above, which was received by it on 25 August 1998 (date of the original deposit)[1]

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion)

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: NCIMB Ltd.,	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorised official(s):
Address: 23 St Machar Drive, Aberdeen, AB24 3RY, Scotland.	Date: 28 August 1998

1 Where Rule 6/4(d) applies, such date is the date on which the status of International Depositary Authority was acquired

Form BP/4 (sole page)

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

Advanced Technologies (Cambridge) Ltd Unit 210 Cambridge Science Park Cambridge CB4 4WA	INTERNATIONAL FORM **VIABILITY STATEMENT** **issued pursuant to Rule 10.2 by the** **INTERNATIONAL DEPOSITARY AUTHORITY** **identified on the following page**

NAME AND ADDRESS OF THE PARTY
TO WHOM THE VIABILITY STATEMENT
IS ISSUED

I DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name AS ABOVE Address:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: NCIMB 40968 Date of the deposit or of the transfer[1]: 25 August 1998

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 26 August 1998 [2]. On that date, the said microorganism was:

☒ [3] viable

☐ [3] no longer viable

1 Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

2 In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.

3 Mark with a cross the applicable box.

Form BP/9 (first page)

IV	CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V.	INTERNATIONAL DEPOSITARY AUTHORITY

Name: NCIMB Ltd ,

Address. 23 St Machar Drive,
Aberdeen,
A24 3RY,
Scotland.

Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorised official(s):

Date: 21 July 1998

4 Fill in if the information has been requested and if the results of the test were negative.

Form BP/9 (second and last page)

CLAIMS

1. A method of transforming trees to modify the fibre characteristics in trees, the method comprising stably incorporating into the plant genome a chimaeric gene comprising a promoter and a nucleic acid coding sequence encoding a gene capable of modifying the extension of fibre cell walls, and regenerating a plant having an altered genome.

2. A method according to Claim 1, wherein said nucleic acid sequence encodes one or more of the class of proteins known as expansins.

3. A method according to Claim 1 or 2, wherein said nucleic acid sequence is derived from *Eucalyptus* or cucumber.

4. A method according to Claim 3, wherein said nucleic acid sequence is one or more of SEQ. ID. Nos. 1-6 hereof or the cucumber expansin sequence known herein as cucumber Ex29 (GenBank Accession No. U30382), or a sequence which has sufficient homology to hybridise to any one of SEQ. ID. Nos. 1-6 or cucumber Ex29 under medium stringency conditions (washing at 2x SSC at 65°C).

5. A method according to any one of the preceding claims, wherein said nucleic acid sequence is an mRNA, a cDNA sequence or a genomic DNA.

6. A method according to any one of Claims 1-5, wherein said chimaeric gene is in accordance with any one of Claims 8-13.

7. A nucleic acid coding sequence encoding a gene capable of modifying the extension of fibre cell walls, the nucleic acid coding sequence being one or more of SEQ. ID. Nos. 1-6 hereof, or a sequence which has sufficient homology to hybridise to any one of SEQ. ID. Nos. 1-6 under medium stringency conditions.

8. A chimaeric gene comprising a promoter and a nucleic acid sequence encoding a gene capable of modifying the extension of fibre cell walls, said nucleic acid sequence being one or more of SEQ. ID. Nos. 1-6 hereof, or a sequence which has sufficient homology to hybridise thereto under medium stringency conditions.

9. A chimaeric gene according to Claim 6 or 8, wherein said chimaeric gene further comprises a terminator.

10. A chimaeric gene according to Claim 6, 8 or 9, wherein said chimaeric gene comprises said nucleic acid coding sequence as said nucleic acid sequence exists in nature, complete with endogenous promoter, terminator, introns and other regulatory sequences.

11. A chimaeric gene according to Claim 6, 8 or 9, wherein said chimaeric gene comprises said nucleic acid coding sequence, with or without introns, combined with a

heterologous promoter, terminator and/or other regulatory sequences.

12. A chimaeric gene according to any one of Claims 6 or 8-11, wherein said promoter is one of said group consisting of the cauliflower mosaic virus 35S promoter (CaMV35S), the cauliflower mosaic virus 19S promoter (CaMV19S), the nopaline synthase promoter, the *rolC*, patatin or *petE* promoters, or the AlcR/AlcS promoter.

13. A chimaeric gene according to any one of Claims 6 or 8-12, wherein said nucleic acid sequence, or parts thereof, is arranged in the normal reading frame direction or in the reverse reading frame direction.

14. A tree comprising a chimaeric gene according to any one of Claims 8-13.

15. A plant cell comprising a chimaeric gene according to any one of Claims 8-13.

16. A tree transformed according to the method of any one of Claims 1-7.

17. A tree according to Claim 16, said tree being a eucalypt, aspen, pine or larch.

18. A seed of a tree transformed according to the method of any one of Claims 1-7.

ABSTRACT

Modification of Plant Fibres

The invention relates to the isolation and characterisation of novel expansin gene sequences from heterologous and homologous tree species and re-introducing such novel genes into trees so as to alter expansin levels. Six novel genes have been identified. Eucalyptus has also been transformed using the cucumber EX29 sequence (GenBank, Accession No. U30382 - known as Cs-EXP1). A change in the plant height and internode length was observed compared with control plants.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANTS:

(A) NAME:	Advanced Technologies (Cambridge) Limited
(B) STREET:	Globe House, 1 Water Street
(C) CITY:	London
(E) COUNTRY:	England
(F) POSTAL CODE:	WC2R 3LA

(ii) TITLE OF INVENTION: Modification of Plant Fibres

(iii) NUMBER OF SEQUENCES: 8

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE:	British American Tobacco (Investments) Limited
(B) STREET:	Regents Park Road
(C) CITY:	Southampton
(D) STATE:	Hampshire
(E) COUNTRY:	England
(F) POSTAL CODE:	SO15 8TL

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE:	Diskette 3.50 inch
(B) COMPUTER:	Viglen P5/75
(C) OPERATING SYSTEM:	MS-DOS Windows 95
(D) SOFTWARE:	Microsoft Word 97

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:	Not yet known
(C) CLASSIFICATION:	Not yet known

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME:	Mrs. M.R. Walford/ Mr. K.J.H. MacLean
(C) REFERENCE:	RD-ATC-19

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE:	01703 777155
(B) TELEFAX:	01703 779856

(2) **INFORMATION FOR SEQ.ID. NO:1**

(i) **SEQUENCE CHARACTERISTICS**

(A) LENGTH:	488 bps
(B) TYPE:	Nucleic acid
(C) STRANDEDNESS:	Double
(D) TOPOLOGY:	Linear

(ii) **MOLECULE TYPE:** cDNA

(vi) **ORIGINAL SOURCE:**

(A) ORGANISM: Eucalyptus grandis

(xi) **SEQUENCE DESCRIPTION:** SEQ. ID. NO:1:

```
ATGGGGGGGG CTTGTGGGTA TGGCAACCTG TACAGCCAAG GCTATGGCAC   50
CAACACTGCA GCTTTGAGCA CTGCCCTGTT CAACAATGGC CTGAGCTGCG  100
GGGCATGTTA CGAGATGCGG TGCAACGACG ACCCCAGGTG GTGCCTCCCG  150
GGGACCATCA TGGTCACGGC AACCAACTTT TGCCCTCCCA ACTTGGCCCT  200
CTCCAACGAC AATTGCGGCT GGTGCAACCC CCCTCTCCAG CACTTCGATA  250
TGGCCGAGCC TGCTTTCTTG CAGATTGCCC AGTACAAAGC TGGGATTGTC  300
CAGGTTTCCT TCAGAAGGGT TCCGTGTGTG AAGAAAGGAG GGGTAAGGTT  350
CACCATCAAT GGGCACTCCT ACTTCAACTT GGTGCTGATC ACCAACGTGG  400
GAGGTGCTGG TGATGTCCAT TCCGTTTCCA TCAAGGGCTC GAGGACTGGT  450
TGGCAAGCCA TGTCAAGGAA CTGGGGCAAA AACTGGCA               488
```

(2) **INFORMATION FOR SEQ. ID. NO:2**

(i) **SEQUENCE CHARACTERISTICS:**

(A) LENGTH:	475 base pairs
(B) TYPE:	Nucleic acid
(C) STRANDEDNESS:	Double
(D) TOPOLOGY:	Linear

(ii) **MOLECULE TYPE:** cDNA

(vi) **ORIGINAL SOURCE:**

(A) ORGANISM: Eucalyptus grandis

(xi) **SEQUENCE DESCRIPTION:** SEQ. ID. NO:2:

```
ATGGGGGGGG CATGCGGGTA TGGCAACCTG TACAGCCAAG GCTATGGCAC     50
CAACACTGCA GCTTTGAGCA CTGCCCTGTT CAACAATGGC CTGAGCTGCG    100
GGGCATGTTA CGAGATGCGG TGCAACGACG ACCCCAGGTG GTGCCTCCCG    150
GGGACCATCA TGGTCACGGC AACCAACTTT TGCCCTCCCA ACTTGGCCCT    200
CTCCAACGAC AATGGCGGCT GGTGCAACCC CCCTCTCCAG CACTTCGATA    250
TGGCCGAGCC TGCTTTCTTG CAGATTGCCC AGTACAAAGC TGGGATTGTC    300
CCGGTTTCCT TCAGAAGGGT TCCGTGTGTG AAGAAAGGAG GGGTAAGGTT    350
CACCATCAAT GGGCACTCCT ACTTCAGCTG TGGTGCTGAT CACCAACGTG    400
GGAGGTGCTG GTGATGTCCA TTCCGTTTCC ATCAAGAGCT CGAGGACTGG    450
TTGGCAAGCC ATGTCAAGGA ATTGA                               475
```

(2) INFORMATION FOR SEQ. ID. NO:3

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	494 base pairs
(B) TYPE:	Nucleic acid
(C) STRANDEDNESS:	Double
(D) TOPOLOGY:	Linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Euclayptus grandis

(xi) SEQUENCE DESCRIPTION: SEQ. ID. NO:3:

```
ATGGGGGGGG CATGTGGTTA CGGGGACCTT CACAGGGCCA CCTATGGCAA   50
GTACAGTGCC GGCTTGAGCT CGATGCTGTT CAACAGAGGG AGTACCTGCG  100
GGGCTTGCTT CGAGCTCCGG TGCGTCGACC ACATTTTGTG GTGCCTCCCT  150
GGTAGCCCGT CGGTGATCCT CACCGCCACC GACTTCTGCC CTCCGAACTA  200
CGGGCTCGCG GCAGATTACG GCGGGTGGTG CAACTTCCCG CAGGAGCACT  250
TCGAGATGTC GGAGGCGGCC TTCGCCGAGA TTGCGGTGCG AAGGGCTGAT  300
GTGGTGCCTA TCCAGTACAG GAGGGTGAAC TGTCTGAGAA GCGGTGGTCT  350
GAGATTCACA TTGAGCGGAA ACTCTCACTT CTTTCAGGTC TTGGTGACGA  400
ATGTAGGCCT AGATGGGGAG GTGATTGCCA TGAAAATGAA GGGATCGAAA  450
ACAGGGTGGA TACCGATGGC AAGAAACTGG GGCAAAAACT GGCA         494
```

(2) INFORMATION FOR SEQ. ID. NO:4

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	437 base pairs
(B) TYPE	Nucleic acid
(C) STRANDEDNESS:	Double
(D) TOPOLOGY:	Linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Eucalyptus grandis

(xi) SEQUENCE DESCRIPTION: SEQ. ID. No. 4

```
ATGGGTTGCC ACCGGGTCCT TGATCCTTTG ATGGCCACGG AGTGCACATC     50
CCCTGCTCCG CCGACATTGG TTATGAGCAC GAGGTTGAAA TAAGAATGGC    100
CGTTGACGGT GAACCGGATC CCTCCGCTTC TCCTGCACCT CACTCTTCGG    150
TAGGCCACCG GGACGATCCC GGCCCTGTAC TGCGCAATGT GCTGGAAGAC    200
CGGCTGGGAG AGGTCGAAAT GGAGTTGAGG AGGGTCGCAC CACCCTCCTG    250
GAGGGCAGAA GTTGGTCGCC GTGACCACAA TGGCGCCCGG GAGGCACCAC    300
TGCGGGTCGT TCACGCACCG GAGCTCAAAG CACGCGCCGC AGCTCAGCCC    350
ATTGTTGAAC AATGCAGTGC TCAGTGCAGC TGTGTTTGTG CCGTACCCTT    400
GGCTGTATAG ATTCCCATAA CCACACGCCC CCCCCAT                  437
```

(2) INFORMATION FOR SEQ. ID. NO:5

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	437 base pairs
(B) TYPE	Nucleic acid
(C) STRANDEDNESS:	Double
(D) TOPOLOGY:	Linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Eucalyptus grandis

(xi) SEQUENCE DESCRIPTION: SEQ. ID. No. 5

```
ATGGGTTGCC ACCGGGTCCT TGATCCTTTG ATGGCCACGG AGTGCACATC     50
CCCTGCTCCG CCGACATTGG TTATGAGCAC GAGGTTGAAA TAAGAATGGC    100
CGTTGACGGT GAACCGGATC CCTCCGCTTC TCCTGCACCT CACTCTTCGG    150
TAGGCCACAG GGACGATCCC GGCCCTGTAC TGCGCAATGT GCTGGAAGAC    200
AGGCTGGGAG AGGTCGAAAT GGAGTTGAGG AGGGTCGCAC CACCCTCCTG    250
GAGGGCAGAA GTTGGTCGCC GTGACAACAA TGGCGCCCGG GAGGCACCAC    300
TGCGGGTCGT TCACGCACCG GAGCTCAAAG CACGCGCCGC AGCTCAGCCC    350
ATTGTTGAAC AATGCAGTGC TCAGTGCAGC TGTGTTTGTG CCGTACCCTT    400
GGCTGTATAG ATTCCCATAA CCACACGCCC CCCCCAT                  437
```

(2) INFORMATION FOR SEQ. ID. NO:6

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	488 base pairs
(B) TYPE	Nucleic acid
(C) STRANDEDNESS:	Double
(D) TOPOLOGY:	Linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Eucalyptus grandis

(xi) SEQUENCE DESCRIPTION: SEQ. ID. No. 6

```
CCTTGACATG GTCTGCCACC TTGTCCGCGA ACCCTTCACG GCGACCGAGT     50
TGACGTTGCC TGCGCCGCCG ACGTTTGTGA CGAGGACGAG CTTGAAGTAT    100
GAGTTGCCGT TGATGGTGAA CCGGATGCCT CCTCTCCTCC TGCACGTCAC    150
CCTCCTGTAC GCAACGTGGA CGATGCCGGC TCGGTACTTG GCAATGTGCT    200
GGAAGACGGG CTGGGAGATG TCGAAGTGGT GTTGGGGCGG GTTGCACCAT    250
CCGCCGGCGT TGTTTGGGAG GGCGTTGTTT GGCGGGCAGA AGTTTGTGGC    300
GGTGACGACG ATGGAGCCGC CCAGGCACCA CTTTCCGTCG TTCACGCACC    350
GGATCTCGAA GCACGACCCC CAGCTCAGCC CGTTTTTTAA CAGCGCCGTG    400
CTCAGCGCCG CCGTGTTCGT ACCGTAGCCC TGGCTGTACA GGTTGCCG      448
```

(2) **INFORMATION FOR SEQ. ID. NO:7**

(i) **SEQUENCE CHARACTERISTICS:**

(A) LENGTH:	19 nucleotides
(B) TYPE	Nucleic acid
(C) STRANDEDNESS:	Single
(D) TOPOLOGY:	Linear

(ii) **MOLECULE TYPE:** Synthetic DNA

(xi) **SEQUENCE DESCRIPTION:** SEQ. ID. No. 7

```
ATGGIGGIGC NTGTGGNTA                    19
```

Key I = Inosine

N = A, G, T, or C

(2) **INFORMATION FOR SEQ. ID. NO:8**

(i) **SEQUENCE CHARACTERISTICS:**

(A) LENGTH:	20 nucleotides
(B) TYPE	Nucleic acid
(C) STRANDEDNESS:	Single
(D) TOPOLOGY:	Linear

(ii) **MOLECULE TYPE:** Synthetic DNA

(xi) **SEQUENCE DESCRIPTION:** SEQ. ID. No. 8

```
TGCCARTTYT GNCCCCARTT                   20
```

Key R = A or G

Y = T or C

N = A, G, T or C

Figure 1

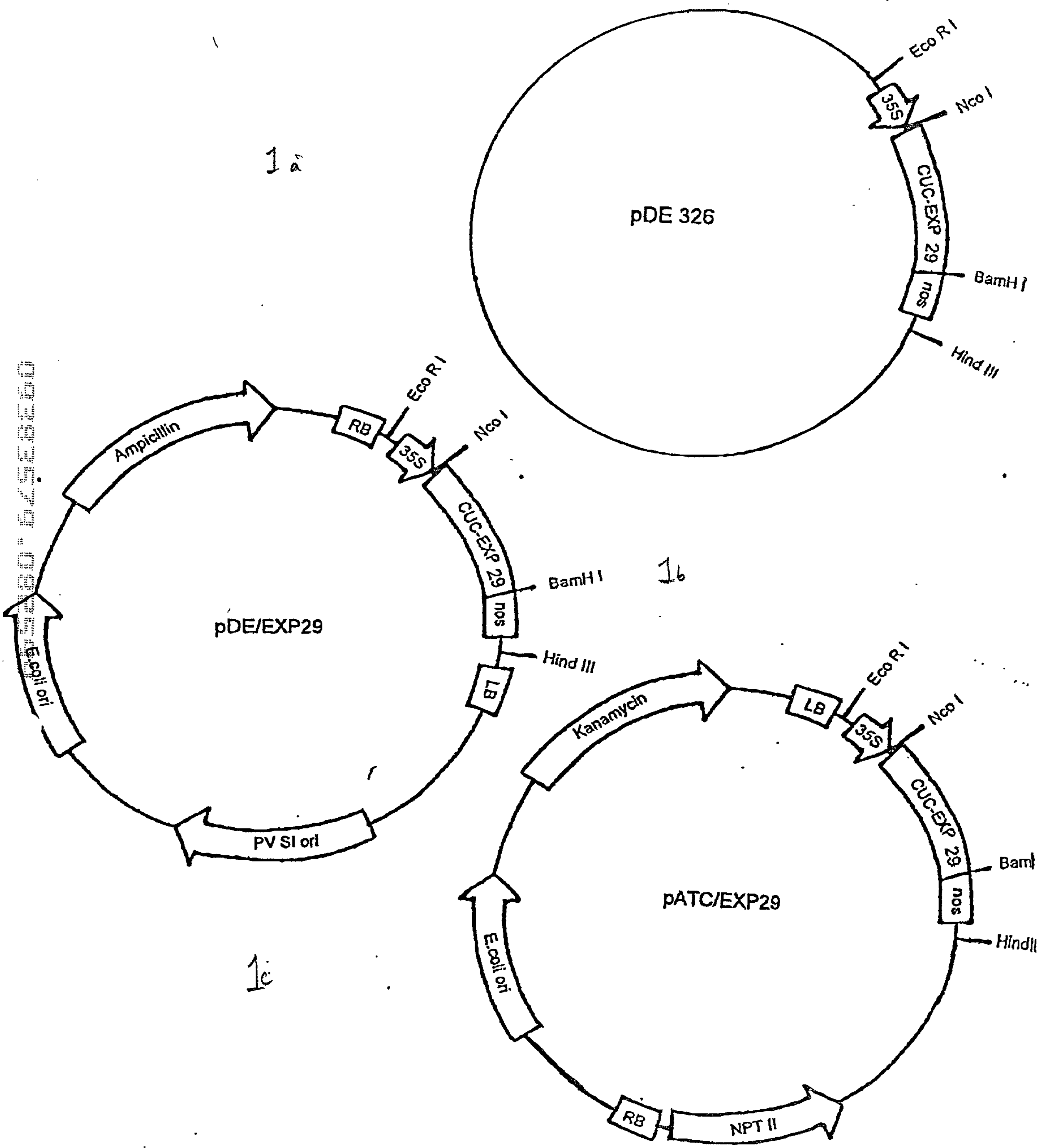
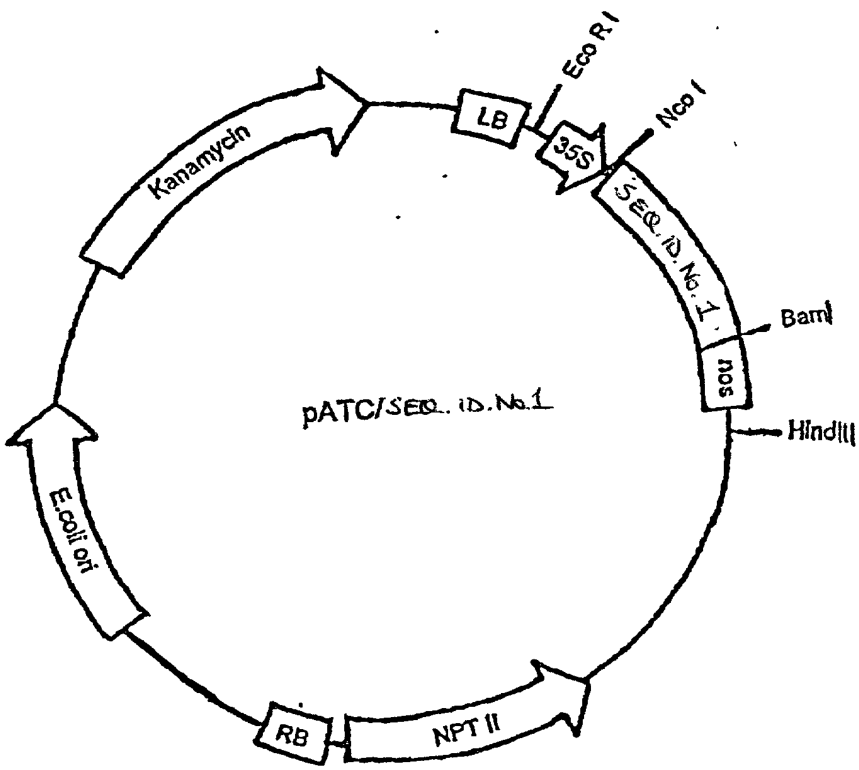

1a
pDE 326
Eco R I
35S
Nco I
CUC-EXP 29
BamH I
nos
Hind III
1b
pDE/EXP29
Ampicillin
RB
Eco R I
35S
Nco I
CUC-EXP 29
BamH I
nos
Hind III
LB
PV SI ori
E.coli ori
1c
pATC/EXP29
Kanamycin
LB
Eco R I
35S
Nco I
CUC-EXP 29
Baml
nos
Hindl
E.coli ori
RB
NPT II

Figure 2


Kanamycin
LB
Eco R I
35S
Nco I
SEQ. ID. No. 1
BamI
nos
HindIII
pATC/SEQ. ID. No.1
E.coli ori
RB
NPT II

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below at 201 et seq. underneath my name.

I believe I am the original, first and sole inventor if only one name is listed at 201 below, or an original, first and joint inventor if plural names are listed at 201 et seq. below, of the subject matter which is claimed and for which a patent is sought on the invention entitled

Modification of Plant Fibres

and for which a patent application:
☒ is attached hereto and includes amendment(s) filed on *(if applicable)*
☐ was filed in the United States on as Application No. *(for declaration not accompanying application)*
with amendment(s) filed on *(if applicable)*
☐ was filed as PCT international Application No. on and was amended under PCT Article 19 on *(if applicable)*

I hereby state that I have reviewed and understand the contents of the above identified application, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED PRIOR TO THE FILING DATE OF THE APPLICATION			
APPLICATION NUMBER	COUNTRY	DATE OF FILING (day, month, year)	PRIORITY CLAIMED
9818808.9	United Kingdom	29 August 1998	YES ☒ NO ☐

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

APPLICATION NUMBER	FILING DATE

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS		
		PATENTED	PENDING	ABANDONED

POWER OF ATTORNEY: As a named inventor, I hereby appoint S. Leslie Misrock (Reg. No. 18872), Harry C. Jones, III (Reg. No. 20280), Berj A. Terzian (Reg. No. 20060), Gerald J. Flintoft (Reg. No. 20823), David Weild, III (Reg. No. 21094), Jonathan A. Marshall (Reg. No. 24614), Barry D. Rein (Reg. No. 22411), Stanton T. Lawrence, III (Reg. No. 25736), Charles E. McKenney (Reg. No. 22795), Philip T. Shannon (Reg. No. 24278), Francis E. Morris (Reg. No. 24615), Charles E. Miller (Reg. No. 24576), Gidon D. Stern (Reg. No. 27469), John J. Lauter, Jr. (Reg. No. 27814), Brian M. Poissant (Reg. No. 28462), Brian D. Coggio (Reg. No. 27624), Rory J. Radding (Reg. No. 28749), Stephen J. Harbulak (Reg. No. 29166), Donald J. Goodell (Reg. No. 19766), James N. Palik (Reg. No. 25510), Thomas E. Friebel (Reg. No. 29258), Laura A. Coruzzi (Reg. No. 30742), Jennifer Gordon (Reg. No. 30753), Jon R. Stark (Reg. No. 30111), Allan A. Fanucci (Reg. No. 30256), Geraldine F. Baldwin (Reg. No. 31232), Victor N. Balancia (Reg. No. 31231), Samuel B. Abrams (Reg. No. 30605), Steven I. Wallach (Reg. No. 35402), Marcia H. Sundeen (Reg. No. 30893), Paul J. Zegger (Reg. No. 33821), Edmond R. Bannon (Reg. No. 32110), Bruce J. Barker (Reg. No. 33291), Adriane M. Antler (Reg. No. 32605), Thomas G. Rowan (Reg. No. 34419), James G. Markey (Reg. No. 31636), Thomas D. Kohler (Reg. No. 32797), Scott D. Stimpson (Reg. No. 33607), Gary S. Williams (Reg. No. 31066), William S. Galliani (Reg. No. 33885), Ann L. Gisolfi (Reg. No. 31956), Todd A. Wagner (Reg. No. 35399), Scott B. Familant (Reg. No. 35504), Warren S. Heit (Reg. No. 36828), Kelly D. Talcott (Reg. No. 39582), and Mark A. Farley (Reg. No. 33170) and, all of Pennie & Edmonds LLP, whose addresses are 1155 Avenue of the Americas, New York, New York 10036, 1667 K Street N.W., Washington, DC 20006 and 3300 Hillview Avenue, Palo Alto, CA 94304, and each of them, my attorneys, to prosecute this application, and to transact all business in the Patent and Trademark Office connected therewith.

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